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SUBSTRATES FOR THE HISTOCHEMICAL LOCALIZATION OF SOME GLYCOSIDASES

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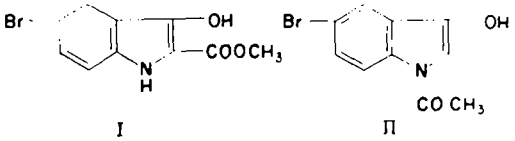
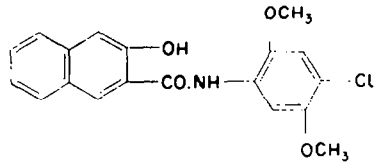
Abstract—Substrates for improved histochemical localizations of some glycosidases were required. The 2-acetamido-2-deoxy-β-D-glucoside, the β-D-glucoside, and the β-D-galactoside of 5-bromoindoxyl were synthesised through 1-acetyl-5-bromoindoxyl and the corresponding acetohalogenosugars. Attempts to prepare the β-D-glucuronide under a variety of conditions were unsuccessful, whereas acetobromo-methyl-glucuronate condensed with naphthoic-2-hydroxy-3-(2′:5′-dimethoxy-4′-chloro-anilide) and a potential histochemical substrate obtained: the preparation of the corresponding derivatives of β-D-galactose and 2-acetamido-2-deoxy-β-D-glucose are also described.

THE histochemical localization of the enzyme N-acetyl-β-glucosaminidase has been studied using the 2-acetamido-2-deoxy-β-D-glucoside of 1-naphthol as a substrate.[1] 5-Bromoindoxyl derivatives have been shown to have advantages in histochemical studies on esterases,[2] and it seemed likely that, provided the appropriate substrates could be prepared, the "indigogenic" principle[3] might be made the basis of improved localizations of glycosidases.



I

II



III

Indican (i.e. 3-β-glucosidoxyindole) has been synthesized[4] from acetobromoglucose through 1-acetyl-3-hydroxyindole or through methyl-3-hydroxyindole-2-carboxylate. Although preliminary experiments[5] indicated that the 2′-acetamido-2′-deoxy-β-D-glucoside could have been obtained through the corresponding indoxylate (I), the route through the 1-acetyl-3-hydroxy-5-bromoindole (II) was preferred since fewer stages were entailed. Acetochloroglucosamine[6] was condensed with the hydroxy

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[2] S. J. Holt and R. F. J. Withers, *Proc. Roy. Soc.* B **148**, 520 (1958).
[3] R. J. Barnett and A. M. Seligman, *Science* **114**, 579 (1951).
[4] A. Robertson, *J. Chem. Soc.* 1937 (1927).
[5] F. B. Anderson and D. H. Leaback, unpublished results.
[6] D. H. Leaback and P. G. Walker, *J. Chem. Soc.* 4754 (1957).

compound (II) under conditions similar to those described previously[4] to give a 29 per cent yield of 1-acetyl-3-(2′-acetamido-3′:4′:6′-tri-O-acetyl-2′-deoxy-β-D-glucosidoxy)-5-bromoindole which was deacetylated with sodium methoxide to give the required 3-(2′-acetamido-2′-deoxy-β-D-glucosidoxy)-5-bromoindole. Under similar conditions the corresponding β-D-glucoside and β-D-galactoside were prepared from the respective acetobromosugars, but attempts to prepare the β-D-glucuronide from acetobromo-[7] or acetoiodo-methyl-glucuronate[8] under a variety of conditions were unsuccessful; no satisfactory explanation of this failure was obvious, but there have been other indications that acetohalogeno derivatives of glucuronic acid are atypical in their reactions. Attempts to prepare 3-β-D-glucuronidoxy-5-bromoindole by catalytic oxidation of the corresponding β-D-glucoside were also unsuccessful.[9]

The failure to prepare indoxyl β-glucuronides, and the advantages of having alternative histochemical substrates available, led to the synthesis of glycosides of other phenols which might give improved histochemical localizations. Phosphates of a series of complex phenols derived from 2-hydroxy-3-naphthoic acid (the Naphthol-AS phenols) have been shown to give good results as substrates for the localization of phosphatases.[10,11] Experiments[12] with several of the Naphthol-AS phenols showed that Naphthol AS-LC (III) [naphthoic-2-hydroxy-3-(2′:5′-dimethoxy-4′-chloroanilide)] combined satisfactorily several properties which were desirable from histochemical considerations; the 2-acetamido-3:4:6-tri-O-acetyl-2-deoxy-β-D-glucoside of this phenol was prepared by a general method previously described,[6] and similar procedures yielded the 2:3:4:6-tetra-O-acetyl-β-D-galactoside and the 2:3:4-tri-O-acetyl-β-D-glucuronide methyl ester from the corresponding acetobromosugars. The 2-acetamido-2-deoxy-β-D-glucoside and the β-D-galactoside were obtained from the polyacetates by catalytic deacetylation from small volumes of methanol.[13] Of the several methods tried for the deacetylation and de-esterification of the 2:3:4-tri-O-acetyl-β-D-glucuronide methyl ester, a barium hydroxide method[14] was preferred.

The use of these compounds in the histochemical demonstration of glycosidase activities is under investigation.

EXPERIMENTAL

Solvents were evaporated at reduced pressure.

1-Acetyl-3-(2′-acetamido-3′:4′:6′-tri-O-acetyl-2′-deoxy-β-D-glucosidoxy)-5-bromoindole (IV)

Acetochloroglucosamine[4] (2·7 g) and 1-acetyl-3-hydroxy-5-bromoindole[15] (1·5 g) were added to acetone (100 ml), cooled (ice-bath), and the suspension gassed for 30 min with a stream of nitrogen. N sodium hydroxide (7·4 ml) was added, and the cold mixture gassed for 1 hr. before the flask was stoppered and left to stand at 5° for 16 hr. Evaporation at room temperature yielded a greenish-brown residue which, after extensive washing with water followed by recrystallization and decolourization from ethanol, gave 1·0 g (29%) of 1-acetyl-3-(2′ acetamido-3′:4′:6′-tri-O-acetyl-2′-deoxy-β-D-glucosidoxy)-5-bromoindole, m.p. 245–246°, $[\alpha]_D^{20}$ −35·5° (*c*, 0·5 in acetone) (Found: C, 49·4; H, 4·7; N, 4·7; $C_{24}H_{27}O_{10}N_2Br$ requires: C, 49·7; H, 4·6; N, 4·8%).

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3-(2′-Acetamido-2′-deoxy-β-D-glucosidoxy)-5-bromoindole (V)

The penta-acetate (IV) (1·0 g) was suspended in dry methanol (50 ml) and N sodium methoxide (1·0 ml) added with shaking; the solid went rapidly into solution, but after a few minutes the de-acetylated compound started crystallizing. The mixture was left at 5° for 16 hr, and the solid filtered and recrystallized (harvesting the crystals as soon as possible to avoid contamination with small amounts of the indigo formed) from hot water to give 0·5 g (70%) of 3-(2′-acetamido-2′-deoxy-β-D-glucosidoxy)-5-bromoindole, m.p. 246–247°, $[\alpha]_D^{19}$ −42·0° (*c*, 0·2 in methanol) (Found: C, 46·6; H, 4·6; N, 6·8; $C_{16}H_{19}O_6N_2Br$ requires: C, 46·3; H, 4·6; N, 6·8%).

3-β-D-galactosidoxy-5-bromoindole

Acetobromogalactose was condensed as for (IV) with the indoxyl compound (II) to give a 40% yield of 1-acetyl-3-(2′:3′:4′:6′-tetra-O-acetyl-β-D-galactosidoxy)-5-bromoindole, m.p. 175–176°, $[\alpha]_D^{20}$ −26·0° (*c*, 1·0 in chloroform). This compound was deacetylated as above; the product did not crystallize from the reaction mixture, but, on evaporation and recrystallization from ethanol/chloroform, yielded 70% of 3-β-D-galactosidoxy-5-bromoindole, m.p. 195°, $[\alpha]_D^{19}$ −70·0° (*c*, 0·4 in ethanol) (Found: C, 44·9; H, 4·5; N, 3·7; $C_{14}H_{16}O_6NBr$ requires; C, 45·0; H, 4·3; N, 3·7%).

3-β-D-glucosidoxy-5-bromoindole

Acetobromoglucose was condensed as for (IV) with the indoxyl compound (II) to give a 15% yield of 1-acetyl-3-(2′:3′:4′:6′-tetra-O-acetyl-β-D-glucosidoxy)-5-bromoindole, m.p. 157–158°, $[\alpha]_D^{20}$ −47·0° (*c*, 1·0 in chloroform) which was de-acetylated as for (V) to give an 80% yield of 3-β-D-glucosidoxy-5-bromoindole, m.p. 259–260°, $[\alpha]_D^{20}$ −77·0° (*c*, 0·25 in methanol) (Found: C, 45·0; H, 4·3; N, 3·5; $C_{14}H_{16}O_6NBr$ requires: C, 45·0; H, 4·3; N, 3·7%).

Naphthalene-2-(2′-acetamido-3′:4′:6′-tri-O-acetyl-2′-deoxy-β-D-glucosidoxy)-3-carboxy-(2″:5″-dimethoxy-4″-chloro-anilide) (VI)

The naphthol derivative (III) was recrystallized from ethanol/chloroform and condensed (4·0 g) with acetochloroglucosamine[4] (4·0 g) in acetone (70 ml) in the presence of N NaOH (13 ml). After solution was complete, the mixture was allowed to stand at room temperature (16 hr) before the acetone was evaporated to leave a yellow residue, which was dissolved in chloroform (200 ml), extracted ten times with 100 ml portions of 5% NaOH and twice with water. The chloroform layer was dried and evaporated to a syrup which crystallized on the addition of ether, and which on recrystallization from ethanol gave 2·4 g (31%) of naphthalene-2-(2′-acetamido-3′:4′:6′-tri-O-acetyl-2′-deoxy-β-D-glucosidoxy)-3-carboxy-(2″:5″-dimethoxy-4″-chloro-anilide), m.p. 192° (softening 182°), $[\alpha]_D^{24}$ −51° (*c*, 1·0 in chloroform) (Found: C, 57·1; H, 5·1; N, 4·0; $C_{33}H_{35}O_{12}N_2Cl$ requires: C, 57·5; H, 5·1; N, 4·1%).

Naphthalene-2-(2′-acetamido-2′-deoxy-β-D-glucosidoxy-3-carboxy-(2″:5″-dimethoxy-4″-chloro-anilide)

The tetra-acetate (VI) (0·8 g) was suspended in dry methanol (6 ml) and N sodium methoxide (0·3 ml) added with shaking. After 16 hr at 5°, the solid was filtered off and recrystallized from 50% ethanol to give 0·5 g (76%) of naphthalene-2-(2′-acetamido-2′-deoxy-β-D-glucosidoxy)-3-carboxy-(2″:5″-dimethoxy-4″-chloro-anilide), m.p. 207°, $[\alpha]_D^{21}$ −76·8° (*c*, 0·5 in 30% dimethylformamide) (Found: C, 57·8; H, 5·2; N, 5·0; $C_{27}H_{29}O_9N_2Cl$ requires: C, 57·8; H, 5·2; N, 5·0%).

Naphthalene-2-β-D-galactosidoxy-3-carboxy-(2″:5″-dimethoxy-4″-chloro-anilide)

Acetobromogalactose was condensed as for (VI) with the naphthol derivative (III) to give a 70% yield of naphthalene-2-(2′:3′:4′:6′-tetra-O-acetyl-β-D-galactosidoxy)-3-carboxy-(2″:5″-dimethoxy-4″-chloro-anilide), m.p. 125°, $[\alpha]_D^{20}$ −75·0° (*c*, 1·0 in chloroform) which was de-acetylated as above and recrystallized from methanol to give a 65% yield of naphthalene-2-β-D-galactosidoxy-3-carboxy-(2″:5″-dimethoxy-4″-chloro-anilide), m.p. 206–207° $[\alpha]_D^{20}$ −87·0° (*c*, 0·25 in ethanol) (Found: C, 56·3; H, 5·4; N, 2·5; $C_{28}H_{36}O_9NCl$. CH_3OH requires: C, 56·5; H, 5·4; N, 2·5%).

Naphthalene 2-β-D-glucuronidoxy-3-carboxy-(2″:5″-dimethoxy-4″-chloro-anilide)

Acetobromo-methyl-glucuronate was condensed as for (VI) with the napthol derivative (III) (but using two equivalents of NaOH) to give a 15% yield of naphthalene-2-(2′:3′:4′-tri-O-acetyl-β-D-glucuronidoxy methyl ester)-3-carboxy-(2″:5″-dimethoxy-4″-chloro-anilide), m.p. 154–155°, $[\alpha]_D^{20}$ −82·0° (*c*, 1·0 in chloroform) which was deacetylated and de-esterfied using barium hydroxide[14] to give a 50% yield of naphthalene-2-β-D-glucuronidoxy-(2″:5″-dimethoxy-4″-chloro-anilide), m.p. 212°, $[\alpha]_D^{20}$ -83·0° (*c*, 0·3 in ethanol) (Found: C, 56·6; H, 4·5; N, 2·7; $C_{25}H_{24}O_{10}NCl$ requires: C, 56·2; H, 4·5; N, 2·6%).

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